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***Spineless Wonders: Welfare and Use of Invertebrates in the Laboratory and Classroom***

**Andrews. Introduction: Laboratory Invertebrates: Only Spineless, Or Spineless and Painless?, pp. 121-125**

Domain 3: Research

Tertiary Species: Invertebrates

SUMMARY: The author of this introduction to the ILAR Journal issue dedicated to Laboratory Invertebrates starts by exploring the ambivalent attitude afforded to invertebrates by us humans. Terms such as "spineless" or "lacking a backbone" can often have a pejorative sense. We tend to consider the invertebrates at the low end of the evolutionary scale and yet, if some declare Octopus vulgaris to be an "advanced invertebrate", how great are then the differences with the "lower vertebrates"?

There are over 1 million invertebrate species, constituting about 95% of all animal species. And yet there is a paucity of information in the area of invertebrate welfare and good experimental practice for invertebrates.

The first piece of legislation affording protection to an invertebrate within the context of experimental research was the 1993 amendment to the UK's Animal (Scientific Procedures) Act. This amendment gave Octopus vulgaris the same legal protection as vertebrates. More recently, the revised EU Directive (2010/63/EU) has granted legal protection to live cephalopods used for scientific purposes. Interestingly, although decapod crustacea (e.g., lobsters, crabs...) had been initially included in the draft, they were finally left out in the approved Directive.

Many questions remain to be explored in relation to the use of invertebrates in research and this issue of ILAR deals with four aspects:

1. Use of invertebrates in biomedical research: this section has a paper by Wilson-Sanders giving a comprehensive overview of the different species used in research and their usefulness in the study of developmental biology, genetics and diseases. Specific examples include: Caenorhabditis elegans (genetic regulation of organ development and programmed cell death); honeybee (organization and elicitation of individual and social behavior patterns); squid (ionic mechanism involved in excitation and inhibition in peripheral and central portions of the nerve cell membrane). Reasons for choosing to use invertebrates in research include: simpler systems to that of the vertebrates; unique or larger structures (such as the giant axon of the squid); unique properties not exhibited by vertebrates (such as pupation and tissue regeneration); lower costs; perception that one is applying a "Reduction" by using less sentient species.

2. Culture and maintenance of invertebrates: Smith and colleagues discuss this aspect and point out that whilst achieving "standardization" in the supply of some invertebrate species such as Drosophila or C. elegans may be relatively straightforward, this is by no means the case for most other invertebrates. These authors also point out that the inclusion of cephalopods in recent EU legislation will require the development of guidelines on all aspects of their maintenance, use and welfare.

3. Evidence of pain and suffering and methods for their alleviation: papers by Elwood and by Crook and Walters explore the differences between nociception and pain perception and call for caution before applying preconceived notions to invertebrate nervous systems which are so different and of which we still know little. There is already a body of evidence for responses to noxious stimuli in the hermit anemone, the California sea slug, the medicinal leech, C. elegans and D. melanogaster. Another paper by Cooper explores the apparent paucity of information on endogenous pathways modulating nociception in invertebrates and, as a consequence, the poorly developed area of anesthesia and analgesia in these species. Systematic reviews of the available literature and the development of techniques for sustained general anesthesia and maintenance of physiological systems (especially in cephalopods) would help advance this neglected area.

4. Attitudes and their influence on regulation and oversight: the philosophical basis for the various attitudes towards invertebrates is discussed by Mather. She describes the contractarian, utilitarian and rights-based approaches to these animals. Finally, Harvey-Clark explores the regulatory and ethical framework that needs to develop for a correct use of invertebrates in the laboratory setting.

In the final section of this introduction the author looks at what is required in the near future when considering the use of invertebrates in research. He points out that the drive towards better, more systematic reporting of studies involving animals (as exemplified by the ARRIVE guidelines) should also apply to studies that involve the use of invertebrates and he advocates the use of the "precautionary principle" to invertebrates in general and to decapod crustacea and cephalopods in particular.

QUESTIONS:

1. What percentage of all animal species are invertebrates?

a. 75%

b. 65%

c. 95%

d. 85%

2. Which of the following species of invertebrate has proved very useful in the study of ionic mechanisms of excitation/inhibition of the nerve cell membrane?

a. Caenorhabditis elegans

b. Squid (Teuthida spp.)

c. Aplysia californica

d. Drosophila melanogaster

3. Which piece of legislation was the first to award protection to one species of invertebrate used for biomedical research:

a. European Directive 2010/63/EU

b. US Guide for the Care and Use of Laboratory Animals 2010

c. UK Animals (Scientific Procedures) Act amendment of 1993

d. Canadian CCAC Guide to the Care and Use of Experimental Animals 1993

ANSWERS:

1. c. 95%

2. b. Squid

3. c. UK Animals (Scientific Procedures) Act amendment of 1993

**Wilson-Sanders. Invertebrate Models for Biomedical Research, Testing, and Education, pp. 126-152**

Domain 3, T3.6/10

Tertiary Species: Invertebrates

SUMMARY: Invertebrates have been used in research and teaching since the late 1800s. Eighteen of the 74 animal based Nobel Prizes for Medicine have been using invertebrates. The fruit fly (*Drosophila melanogaster*) chromosomes have been mapped and sequenced. This has been used to study visual and behavioral pathways, virus and host genetic relationships, embryonic patterning, aging, substance abuse, hematopoiesis, metabolic diseases such as diabetes and obesity, and development of models for human disease such as Alzheimer’s, Parkinson’s, and Huntington’s. Due to its similarity to vertebrate cardiogenesis, it has been used as a model for cardiac development and disease. The fly has also been used for eye development studies and renal development and differentiation. *Caenorhabditis elegans* (the worm) is prolific and easily grows in the laboratory. Its nervous system is fully mapped and serves as a model for Parkinson’s, Alzheimer’s, and Huntington’s disease, aging, apoptosis, diabetes, cancer, immune disorders, space biology, substance abuse, and development of therapeutic agents. *Drosophila* and *C. elegans* are the most often used invertebrates in research. Grasshoppers (*Dissosteira carolina*) have been used in neural development. The sea squirt (*Ciona intestinalis*) has been used for animal development and adaptive immunity. The sea star (*Asterias forbesii*) has been used in reproductive development research. The starlet sea anemone (*Nematostella vectensis*) has been proposed to be used in molecular and evolutionary biology. *Daphnia* has been used to study host-parasite interactions. Gastropod mollusks, such as the pond snail (*Lymnaea stagnalis*) have been used in neuronal studies of learning and memory. Honeybees (*Apis mellifera*) have been used to study behavior and communication, memory, and sensory processing. Yucatan octopuses (O. maya) are models for comparative psychology and neurobiology, and neural electrophysiology, neurochemistry, and neurosecretion. Luciferase has been studied in the firefly or lightning beetle (*Photinus pyralis*). Invertebrates have been used for a wide range of medicinal properties, from anticoagulants, antimicrobials, immune protectors, pain alleviation, sunscreens, and antioxidants. Spongin, the protein that makes up the skeleton of sponges have been used in osteoarthritis and other degenerative bone diseases. Invertebrates have also become a good vertebrate substitute in teaching settings. Invertebrate models provide good opportunities for practicing ethical care and meeting one of the Three R’s ‘replacement.’

QUESTIONS:

1. What are the 2 most commonly used invertebrate species used in research?

2.  Due to its similarity to vertebrate cardiogenesis, which invertebrate species has been used as a model for cardiac development and disease?

3.   What species has been used to study behavior and communication, memory, and sensory processing?

ANSWERS:

1.   Fruit fly *Drosophila* *melanogaster* and the worm *Caenorhabditis elegans*

2.  Fruit fly *Drosophila melanogaster*

3.  Honeybees *Apis mellifera*

**Smith et al. Culture and Maintenance of Selected Invertebrates in the Laboratory and Classroom, pp. 153-164**

Domain 3: Research

Tertiary Species: Invertebrates

SUMMARY

Introduction: Invertebrates; models for cancer, aging, immunology, endocrinology, toxicology, developmental biology, tissue regeneration, genetics, molecular biology, learning and memory and biomimetics.

Classroom Use: Primary and secondary school; comparative anatomy, developmental biology, genetics, behavior, evolution, ecology, conservation

Public Aquariums And Zoos: Displays of aquatic invertebrates as well as ant colonies, bee hives, butterfly houses, and arachnid and insect collections

1.   *Caenorhabditis elegans*

* Free-living nematode
* Uses include developmental biology, neurobiology, cell death, anatomy (nematode is transparent)
* Genome first to be completely sequence for a multicellular organism
* Anatomy and Biology
* Unsegmented nematode, about 1 mm length
* Completes life cycle in ~5 days at room temp 22C if enough food
* Worms male, or more commonly, hermaphroditic
* Offspring of self-fertilized worms usually genetically identical to each other and the parent
* Culture - Preferred food source is bacterial lawn of E coli
* Health Challenges: Bacterial and fungal contamination of stock

2.       *Aplysia californica*

* California sea hare = opisthobranch mollusc
* Uses include developmental and neurological studies and for learning studies on; habituation, sensitization and classical conditioning
* Anatomy and Biology
* Herbivorous marine gastropod, Pacific Ocean off coast California
* Only small vestigial shell, protects heart and organs
* 2 large ventrally placed tentacles, and 2 sensory tentacles called rhinophores on top of anterior portion of head, with tactile and chemoreceptors
* Breathes by directing water over gills, using modified flaps (parapodia) on back or mantle
* Water goes over gills, then expelled through posteriorly directed tube called a siphon
* When disturbed, can retract siphon, but can modify behavior
* Ink gland just under the shell- releases when threatened, color of ink and animal derived from pigments in algae consumed
* Hermaphroditic, but does not self fertilize.
* Alternates between male and female
* Lays several thousand eggs
* Adult at 94 days, then live an additional 6-10 months
* Adult weight around 500- 1000 gm
* Housing and Diet
* Glass, fiberglass or plastic tanks
* Water quality critical
* Natural or artificial seawater, salinity at 30-36 ppt, temp 13- 16C
* pH between 8- 8.6, Oxygen at least 95%
* Fed to satiation q 3 days, marine macroalgae, red algae

3.       *Drosophila melanogaster*

* Fruit fly, research organism for almost a century
* Originally, genetic inheritance
* Current; genetics, also molecular and developmental, embryonic development, and adult structures in pupal stage
* Insect = Exoskeleton
* Body with 3 segments; head, thorax, abdomen
* Sensilla are small hairs all over t the body, that act as sense organs and tell about touch, taste, smell and sound
* Life cycle 12-14 d
* Fertilized eggs deposited on food source, hatch in a day, larvae molts 3X, pupates
* Sexually dimorphic, females larger
* Housing and diet
* Commercially available fly culture jars, commercial fly growth medium
* Flies incubated at room temp, then transferred to new vials prn
* Culturing and genetic breeding
* To separate adult flies based on phenotype or sex, anesthetize w chloroform , ether, or by chilling, or CO2
* Petri plate under dissecting microscope, chilled
* Challenge - contamination with bacteria or fungus and infestation with mites

4.  Hermit Crabs

* Mainly pets, display in classroom
* Research in behavior, competition and population studies
* 2 categories; marine/ aquatic and terrestrial
* 4 families of aquatic hermit crabs; Diogenidae, Paguridae, Pylochelidae and Pylojacquesidae
* Terrestrial hermit crabs; Coenobitidae and Parapaguridae
* More than 600 spp hermit crabs, most marine
* Most lab animal hermit crabs are land hermit crabs especially Coenobita
* Anatomy and Biology
* Hermit crabs aren’t true crabs since they lack a complete exoskeleton
* Molt as they develop
* Nocturnal, social
* Life span up to 30 years wild and 15 years in captivity
* Terrestrial hermit crab
* Glass aquarium with ventilated lid, substrate sand, wood chips or coconut fiber.
* Crab buries itself to molt
* Land crabs require chlorine-free water and saltwater
* Omnivores and scavengers, fed fruit, vegetables, meat, and commercially available pelleted food.
* Temp and humidity levels are critical.
* Modified gills that allow them to breathe air, Humidity between 70- 80%
* Health Challenges: Mainly husbandry, aggression from tank mates
* Limbs regenerate usually when crab molts
* Infestation by various species of mites

5.   Horseshoe crab - *Limulus polyphemus*

* Survived essentially unchanged for more than 200 million years
* Closest living relatives of trilobites
* More closely related to scorpions and spiders than to true crabs
* 4 species; American horseshoe crab, Limulus polyphemus, in Atlantic coast of North America from Maine to the Yucatan peninsula
* Other 3 species, Tachypleus tridentatus, T. gigas and Carcinoscorpius rotundicauda
* Unique marine invertebrate with multiple commercial uses
* Fertilizer, livestock feed, bait in commercial whelk and ell fisheries,
* Exhibit animal in aquaria and classroom
* Research animal model; Embryology, physiology and function
* Primary source of Limulus amebocyte lysate, widely used to detect endotoxins on or in medical devices, implants and vaccines
* Anatomy and biology
* Body 3 sections; frontal prosoma (cephalothorax) with an anterior flange, hind body opisthosoma (abdomen) and posterior telson (tail)
* Pair of laterally compound eyes
* 5 pairs of book gills that contain gill leaflets used for respiration, osmoregulation and propulsion.
* Like most invertebrates, has Open circulatory system with hemolymph, containing a copper-based hemocyanin for oxygen transport
* Lifespan approximately 18- 22 years
* Adequate mechanical and biological filtration essential
* Extremely tolerant of wide range of environmental conditions
* Natural or commercial saltwater
* Diet in Captivity - live brine shrimp, dead fish, squid, crabs, clams, frozen bring shrimp, commercial artificial shrimp and fish diets
* Health Challenges
  + Noninfectious - husbandry
  + Infectious - Gm negative bacteria, parasites, allege, fungus, cyanobacteria, shell lesions
* Euthanasia via IV pentobarb in dorsal cardiac sinus

6.  Cephalopods

* Research- neurobiology and behavior; sensory perception, angular acceleration, vision, central and peripheral nerve conduction, neurotransmitters, skin cells
* National Resource Center for Cephalopods
* Cephalopod: 2 subclasses; Coleoidea (octopus, squid, cuttlefish) and Nautiloidea
* Semelparous - grow rapidly to sexual maturity, breed once then die
* Skin extremely fragile
* Well-vascularized gills in the mantle
* Hemolymph - blue from copper-based hemocyanin
* Most evolved nervous system of all invertebrates
* Copper toxic to all invertebrates
* Requires challenging and precise marine habitat
* Diet - live prey items, fresh frozen,
* Health Challenges: Poor water quality, trauma, abrasions on skin leading to secondary bacterial infections, septicemias, cannibalism
* Anesthetics - magnesium chloride in seawater, benzocaine
* Most cephalopods life span only 12- 14 months, except nautilus up to 20 years)

QUESTIONS:

1.  Which multicellular organism was the first to have its genome completely sequenced?

a.  Drosophila melanogaster

b.  Caenorhabditis elegans

c.  Limulus polyphemus

d.  Aplysia californica

2.  From what species is the LAL assay derived?

a.  Leucothrix

b.  Limulus polyphemus

c.  Liponyssoides spp

d.  Lolliguncula brevis

3.  Define semelparous.

ANSWERS:

1.  *Caenorhabditis elegans*, a free-living unsegmented 1 mm long nematode

2.  LAL= Limulus amebocyte lysate, from the American horseshoe crab, *Limulus polyphemus*.  Used to detect endotoxins on or in medical devices, implants and vaccines

3.  Semelparous - species grows rapidly to sexual maturity, breeds once, then dies

**Smith. Invertebrate Resources on the Internet, pp. 165-174**

SUMMARY: Invertebrates are extremely diverse organisms, ranging from simple sponges to complex cephalopods, and have played a major role in exhibits or displays at most aquaria, zoos and nature centers. They are also used for a variety of research purposes, from basic biological studies to investigations of mechanisms of cell signaling to experimental models of cancer and human disease. In class room, invertebrates have been used for teaching zoology, anatomy, physiology, embryology, genetics, husbandry and conservation to primary, secondary and college students. There are number of Invertebrate resources on the Internet. These resources should be used as guide for locating preliminary information. The information consists of taxonomy, natural history, husbandry, culture, welfare, anatomy and histology, physiology, ontogeny, genetics, conservation, toxicology, educational resources, list servers, and databases.

The internet provides information about the following:

General information: brief overview of all invertebrate phyla with links to information on specific groups

Basic information on the Major invertebrate phyla:

* Porifera: introduction to Porifera, with e.g. of sponge types, taxonomy, anatomy of sponges
* Cnidaria (corals, anemones, box jellies, hydroids, and jellyfish): basic characteristics of this phylum, list of scientist working on hydrozoans and links to other web sites on hydrozoans, resources describing biodiversity in the Scyphozoa, resources for methods and links related to the taxonomy, biogeography, habitat characteristics, and environmental correlates of Hexacorallia, and information about coral reefs.
* Ectoprocta (bryozoans and Marine mats): interactive global reference for the collection, preservation, and dissemination of scientific knowledge on marine, estauarine, and fresh water bryozoans.
* Platyheminthes (flatworms, trematodes, and cestodes): general information and general characteristics
* Annelids (ragworms, earthworms and leeches): introduction to annelids, links to history, evolution, systematic, and morphology of this phylum, annelid taxonomy and collections, general information on earthworms (anatomy, reproduction, and behavior)
* Echinodermata (starfish, brittle stars, sea urchins and sea cucumbers): general info, characteristics and examples, list of researchers, publications, theses etc.
* Molluscs (snails, octopuses, squid, clams, scallops, oysters, and chitons): general background info on phylum, info on evolution, images of selected species, anatomy, feeding and reproduction.
* Arthropods (insects, arachnids, and crustaceans): general overview, bibliographies, collections and data bases, publications, and research activities, research institutions

Invertebrate paleontology: info on collection, preservation, and interpretation of invertebrate fossils sponges, bryozoans, corals, molluscs, arthropods, and brachiopods, images of invertebrate fossils from various research collections.

Information about invertebrates commonly used in Research, teaching and display: general information on biology, ecology, and laboratory uses.

The Nematode (C. elegens): Databases of behavioral and structural anatomy, with detailed illustrated atlas of C. elegans anatomy, info on genetics and genomics, inventory of genes and their variants of C. elegans, educational portal for uses of C. elegans in teaching and research

Information on:

* California Sea Hare (*Aplysia californica)*
* Fruit Fly (Drosophila melanogaster)
* Hermit Crabs
* Horseshoe Crab (*Limulus polyphemus*)
* Cephalopods

Information on Invertebrates Associations and Societies

QUESTIONS:

1. T/F: The use of invertebrates in research labs and in teaching has decreased over the past 20 years.

2. T/F: Invertebrates are used for variety of research purposes, from basic biological studies to investigation of mechanisms of cell signaling.

3.  What is the *scientific name* for fruit fly?

4.  What is the *scientific name* for California Sea Hare?

5.  What is the *scientific name* for Horseshoe Crab?

ANSWERS:

1.  F

2.   T

3**.**Drosophila melanogaster***.***

4. *Aplysia californica*

5.   Limulus polyphemus

**Elwood. Pain and Suffering in Invertebrates?, pp. 175-184**

Domain 2: Management of Pain and Distress; Task T1: Recognize pain and/or distress; Knowledge Topic TT2.1 Anatomy and physiology of pain and distress as they pertain to laboratory animals

Tertiary Species: Invertebrates

SUMMARY

Defining Pain Versus Nociception: Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. Nociception includes the neural processes of encoding and processing noxious stimuli, or the detection and reaction “to stimuli that may compromise their integrity. Nociception is central to the concept of pain, as without it the experience of pain is unlikely. The demonstration of nociceptors presence does not indicate the capacity to experience pain.

How Can Pain Be Identified In Invertebrates? Following criteria that are similar to previous works in mammals, amphibians, fish and various invertebrates. Assessing the presence of suitable receptors and a suitable central nervous system, responsiveness to opioids, analgesics and anesthetics; physiological changes; avoidance learning; protective motor reactions; tradeoffs between stimulus avoidance and other activities; and cognitive ability and sentience.

Suitable Receptors: There is a wide taxonomic occurrence of functional nociceptors so the demonstration of their presence does not indicate the capacity of experience pain in invertebrates.

A Suitable Central Nervous System: A limited investigation in central nervous system of invertebrates does not provide clues about pain experience.

Responsiveness To Opioids, Analgesics and Local Anesthetics: The use of these analgesics in invertebrates might indicate a central modulation of responses but potential peripheral effects may explain the analgesia.

Physiological Changes: Might prove useful for answering the question of pain in invertebrates, however to date, the study of their appearance in invertebrates is limited and reveals little about their pain experience.

Avoidance Learning: A rapid avoidance learning, coupled with prolonged memory, indicates central processing and is consistent with pain.

Protective Motor Reactions: Grooming or rubbing might indicate an awareness of the specific site stimulus application and seems to be more than a reflex reaction in invertebrates.

Tradeoffs Between Stimulus Avoidance And Other Activities: Tradeoffs with other motivational systems indicate central processing and may be useful to determine what invertebrates will “pay” to avoid the noxious stimulus.

High Cognitive Ability, Consciousness, And Sentience: An ability to use information from various sources might indicate sufficient cognitive ability in invertebrates to have a fitness benefit from pain experience.

Conclusion: Behavioral studies are consistent with the idea that some invertebrates such as crustaceans and molluscs experience pain. While awaiting the results of further relevant studies, perhaps all who use invertebrates should consider the possibility that at least some might suffer pain and, as a precaution, ensure humane care these animals.

QUESTIONS:

1. True or False. The sensory systems that respond to noxious stimuli and mediate protective reflexes are termed nociceptors.

2. True or False. Empathy for invertebrates is typically low and some researchers believe that it would be “inconvenient” if these animals were believed to feel pain.

3. True or False. Nociceptive abilities are found in most of the major animal phyla and thus are presumably a product of very early evolution.

4. Substantial research in invertebrates, on various taxa, necessary to assess experience pain should show:

a. Rapid avoidance learning of noxious stimulus.

b. Prolonged responses directed to the specific site on their body where the noxious stimulus was applied.

c. Tradeoffs between avoidance and other activities that would indicate central decision making rather than reflex reaction.

d. All of them are right.

5. True or False. Available data are not consistent with the idea of pain in some invertebrates.

ANSWERS:

1. True

2. True

3. True

4. d

5. False

**Crook and Walters. Nociceptive Behavior and Physiology of Molluscs: Animal Welfare Implications, pp. 185-195**

Domain 2: Management of Pain and Distress; Task T1: Recognize pain and/or distress; Knowledge Topic TT2.1 Anatomy and physiology of pain and distress as they pertain to laboratory animals

Tertiary Species: Invertebrates

SUMMARY: The authors review current literature pertaining to the physiology of nociceptors and the behavioral responses to stimulation of those nociceptors in several taxa of molluscs that are commonly used in research. Studies of nociceptive physiology in molluscs have revealed a wealth of knowledge that contributes to the understanding of the mechanisms of pain in humans. However, these studies raise the concern that the molluscs used in the studies may be experiencing pain as well, which is a major animal welfare concern. Nociception is defined as the detection of stimuli that are injurious or would be if sustained or repeated. Detection of these injurious stimuli occurs through primary sensory neurons that are preferentially sensitive to noxious stimuli, called nociceptors. A wealth of research suggests that nociceptive processes in primary nociceptors are highly conserved across taxa, including molluscs. Pain, as experienced by humans, is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. Due to the fact that the definition of pain has an emotional component, and the difficulty in clearly identifying this component in other species, it is important to distinguish between the sensory neuron activation and emotional perception when considering studies performed in animals. Behavioral studies of responses to noxious stimuli in molluscs primarily use gastropods (i.e., snails, slugs, limpets, etc.) and cephalopods (i.e., squid, cuttlefish, octopus, etc.). These studies have consistently demonstrated behavioral responses that would effectively avoid continued stimulation of nociceptors, including reflexive withdrawal from potentially threatening stimulus, rapid escape from the stimulus, and prolonged recuperative behaviors. In addition, some studies have provided evidence of emotion-like reactions to stimuli in molluscs. Studies in Aplysia demonstrated the development of a conditioned fear-like state when food was presented after previously being presented with food and a shock concurrently. While such studies do not provide conclusive evidence that molluscs experience pain similar to the human experience, the evidence does indicate that exposure to noxious stimuli results in behavioral responses to escape and avoid those stimuli. To preserve animal welfare in molluscs, the authors recommend the use of an appropriate anesthetic, such as magnesium chloride, whenever any procedures will be performed that produces tissue damage or possible stress.

QUESTIONS:

1.  T/F. Nociceptors are primary sensory neurons that are preferentially sensitive to noxious stimuli.

2.   T/F. Nociceptive processes in primary nociceptors are not conserved across taxa

3.   Behavioral studies of responses to noxious stimuli using molluscs primarily use which classes of molluscs?

4.   What is the anesthetic of choice for use in gastropods?

ANSWERS:

1.  True

2.  False

3.   Cephalopods and gastropods

4.   Isotonic magnesium chloride solution

**Cooper. Anesthesia, Analgesia, and Euthanasia of Invertebrates, pp. 196-204.**

Domain 2: Management of Pain and Distress

Tertiary Species: Invertebrates

SUMMARY: There at least 1 million species of invertebrates, the majority being ectotherms. There is debate as to whether they can feel pain, although most species do respond to adverse stimuli. Many have a well-developed nervous system, and in some, an opioid system similar to mammals has been identified.

Isoflurane (5-10%, agent of choice), sevoflurane, halothane (5-10%), or carbon dioxide (10-20%) can be used for inhalation anesthesia of terrestrial species. These are generally safe and many invertebrates are tolerant to hypoxia. They can be administered in a jar or an anesthetic chamber. It is important to place gauze over the end of the tubing so the invertebrate does not escape. Induction can take several minutes and recovery can be prolonged (2-5 hrs). Water-soluble agents should be used for terrestrial or amphibious molluscs.

For aquatic species anesthesia, MS 222, benzocaine, carbon dioxide and various other agents can be used. A container with water from the animal’s tank or fresh water of the same temperature can be used. With MS 222 and benzocaine, the animal, once immobile, can be removed from the water for 10-15 minutes but must be kept wet. Recovery usually takes about 30 minutes or more. Carbon dioxide is reliable and inexpensive. It can be bubbled from a cylinder through water (or diluted with soda water) in a suitable container. Other agents include chloretone, ethanol, chloroform, and magnesium chloride.

It is difficult to assess depth of anesthesia in invertebrates. Immobility and loss of a righting reflex are good indicators of full anesthesia. If a terrestrial vertebrate has a prolonged recovery, it should be returned to the anesthetic chamber for 10-30 minutes of pure oxygen and failure of signs of recovery after 12 hours is an indication of death. In aquatic species, the oxygen should be bubbled through the water continuously for 10-20 minutes or intermittently for 1-2 hrs. Maintenance of fluid balance is important as most invertebrates have a large surface area in proportion to body mass. However, this is less important in aquatic species.

There is limited information on euthanasia of invertebrates. Physical methods include decapitation and chemical methods include injection of pentobarbitone and immersion in 70% ethanol.  Rapid freezing compromises histological examination.

QUESTIONS:

1. What is the scientific name for honey bees?
   1. *Drosphilia melanogaster*
   2. *Apis mellifera*
   3. *Helix aspersa*
   4. *Charybdis feriata*
2. T/F: In invertebrates, hypothermia can be used for surgical or other invasive procedures.
3. T/F: Twitching of limbs, antennae, and palps and the presence of muscle tone are reliable signs for assessing depth of anesthesia in invertebrates.

ANSWERS:

1. *Apis mellifera*
2. F
3. F

**Mather. Philosophical Background of Attitudes toward and Treatment of Invertebrates, pp. 205-212**

Domain 3: Research

Tertiary Species: Invertebrates

SUMMARY: There are three different philosophical approaches that people take when forming their attitude towards invertebrates: contractarian, utilitarian, and right-based. The contractarian approach presumes complete separation of humans and animals, and that animals are only automata. It states that we should evaluate the effects of interactions with animals for our own good, because cruelty and neglect demean us. The utilitarian approach is an objective way of looking at animals, where humans should assess gains and losses when regarding animals and invertebrates should be respected and protected based on their sheer number and therefore critical to life on the planet. The utilitarian approach takes pain into account in the gain-loss equation, and the assessment of pain vs. nociception. The rights-based approach assesses the quality of life of each animal-human interaction and takes into account self-awareness and consciousness. The rights-based approach concentrates on the usefulness of enrichment in the animals' everyday lives. Regardless of the approach, information and education about the animals are critical to understanding how they ought to be treated.

QUESTIONS:

1. Which approach states that we should evaluate or effects of our interactions with animals not necessarily for the animals' good, but because we should not participate in cruelty and neglect?

a. Contractarian

b. Utilitarian

c. Rights-based

d. None; there is not part of a philosophical view

2. T or F: Information and education about the animals should not influence how we treat animals or what our philosophical approach is.

3. Which philosophical approach has little to no bearing on understanding how animals should be treated?

a. Contractarian

b. Utilitarian

c. Rights-based

d. They all have bearing

ANSWERS:

1. A

2. F

3. d

**Harvey-Clark. IACUC Challenges in Invertebrate Research, pp. 213-220.**

Domain 5: Regulatory Responsibilities; T2. Advocate for humane care and use of animals T6. Review protocols and provide advice to investigators and the IACUC

Tertiary Species: Invertebrates

SUMMARY: Invertebrates represent the largest number and greatest diversity of all animals used in research. Fish and invertebrates are excluded from the Animal Welfare Act, although PHS-approved research protocols require ethical justification for all animal use. The review of invertebrate policies differs from institution to institution. Some will review invertebrate protocols, others will not if the invertebrate does not meet the legal definition of “animal”.

Many invertebrates are at-risk, threatened, or endangered species, as defined by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Federal and states may have additional layers of permitting.

Particular challenges to the IACUC, due to the sheer diversity of the phylum, include: sourcing, record keeping, counting, hazard prevention when working with toxic or injurious species, housing, containment, food, veterinary care, invasive procedure evaluation, and treatment options.

The most notable debate currently is the issue of pain and suffering. Many species of invertebrates have the capacity for nociception and withdraw from damaging stimuli. However, the presence of nociception does not conclusively demonstrate that invertebrates are capable of experiencing the mental/emotional state of pain that is acknowledged in many vertebrate species. None of the evidence is conclusive in either confirming or refuting the possibility of pain and distress homologues in invertebrate species.

QUESTIONS:

1. How does the 8th edition of the Guide deal with invertebrates?

2. Do the 2007 AVMA Guidelines for Euthanasia include techniques and ethics for invertebrate species?

3. Which regulatory agency oversees the importation/exportation and interstate trade of wild invertebrates and their tissues?

ANSWERS:

1. It does not address it in detail, but establishes general principles and ethical considerations that are also applicable to these species and situations

2. No

3. US Fish and Wildlife Service